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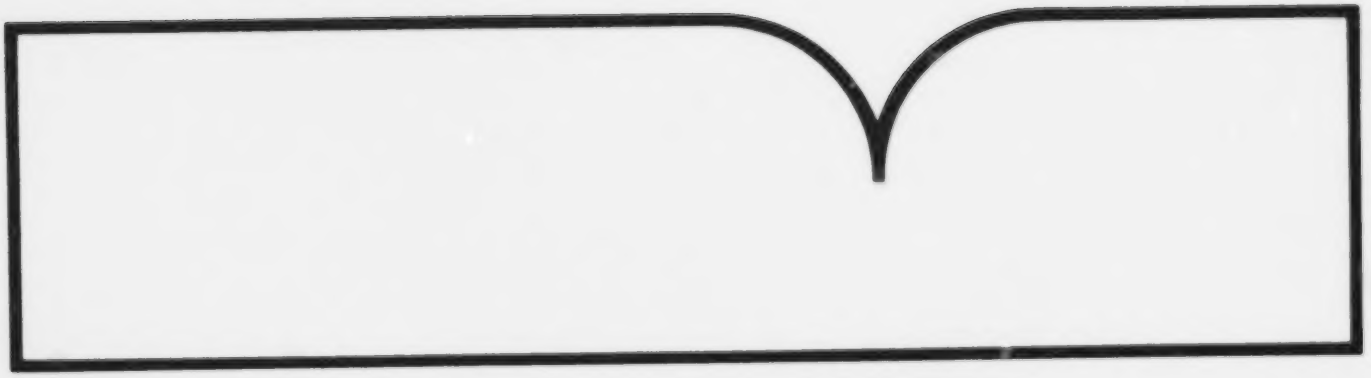
Carbaryl: Decision Document

(U.S.) Environmental Protection Agency
Washington, DC

Dec 80



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The Position Document addresses the risks and benefits of pesticide products containing the subject active ingredient. The Agency has determined that the use of products containing the subject active ingredient may meet or exceed a risk criterion described in 40 CFR Part 154. Potential hazards will be examined further to determine the nature and extent of the risk, and considering the benefits of the subject active ingredient, whether such risks cause unreasonable adverse effects on the environment.

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CARBARYL: DECISION DOCUMENT

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460

DECEMBER 1980

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CARBARYL: DECISION DOCUMENT

I. INTRODUCTION

Carbaryl is a widely used pesticide which is manufactured in the United States by the Union Carbide Corporation under the trade name "Sevin." Carbaryl is a member of the carbamate family of pesticides, and like other family members, derives its efficacy from its ability to inhibit the enzyme cholinesterase. The inhibition of this enzyme renders the nervous system inoperable, thereby killing the target pest. In general, N-methyl carbamates have been found to be more efficacious than N-dimethyl carbamates. Carbaryl belongs to the N-methyl carbamate class of insecticide. Two distinct characteristics have made carbaryl the most popular carbamate the world over. These characteristics — low mammalian oral and dermal toxicity, and a rather broad spectrum of insect control — have led to its widespread use in a wide range of applications.

Carbaryl use was first called into question in 1968, after a study by Smalley et al. (1968) found carbaryl to be a potent teratogen when administered in low doses to pregnant beagle dogs. A 1969 study of beagles (Imming et al., 1969) commissioned by the Union Carbide Corporation also showed positive results. Since then, carbaryl has been the subject of numerous studies of its effects on a number of mammalian species.

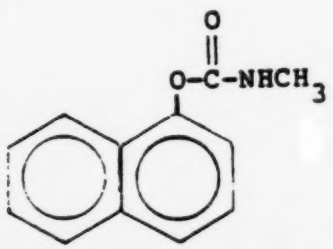
This position document summarizes the results of the Agency's review of currently available toxicological evidence on carbaryl as it pertains to the potential human health hazards of the pesticide. The document is comprised of five sections. Section I is this introduction. Section II discusses general

products, uses, production, and tolerances. Section III addresses the primary purpose of the review: it compares data on potential adverse effects of carbaryl to humans with the Agency's criteria for a Rebuttable Presumption Against Registration. Section IV summarizes the conclusions of this review of carbaryl and recommends several actions to be taken in response to these conclusions. Section V is a bibliographical listing of works cited.

II. GENERAL INFORMATION

A. Chemical Identity

The chemical name for carbaryl is 1-Naphthyl methylcarbamate or 1-Naphthyl N-methylcarbamate. The Chemical Abstracts Service (CAS) has assigned to carbaryl the registry number 63-25-2. The chemical formula is $C_{12}H_{11}NO_2$ with a molecular weight of 201. The structural formula of carbaryl is shown below:



The pure form of carbaryl is a white crystalline solid. The technical product is pink, lavender, or tan in color. It is essentially odorless and melts at 142°C. The vapor pressure is less than 0.005 mm Hg at 26°C. Carbaryl is slightly soluble (40 ppm) in water at 30°C. It is fairly soluble in certain polar organic solvents, including acetone, cyclohexanone and dimethyl formamide. It is slightly soluble in hexane, benzene, and methanol.

B. Registered Products, Uses, and Tolerances

1. Products and Production. Carbaryl has been registered for pesticidal use since 1959. Carbaryl is used in 1,518 federally registered products, made by 295 registrants. Production and consumption in the United States in 1972 were estimated at 53 and 25 million pounds, respectively, an estimated 28 million pounds having been exported. In terms of general use categories, the pattern of 1972 domestic consumption was roughly as follows (approximations expressed in millions of pounds): agriculture - 19, home and garden - 3.5, government - 1.5, industrial - 1.0 (Von Rumker et al., 1974). Production for domestic consumption in 1979 has been estimated to be between 15 million to 25 million pounds.

2. Uses. Carbaryl is registered as an insecticide/acaricide, as well as a plant regulator used in thinning apples. Its major uses include applications to cotton, peanuts, soybeans, field and sweet corn, ornamentals and turf, forest and shade trees, deciduous tree fruits, many other fruit, vegetable, and nut crops, poultry and pets (Von Rumker et al., 1974). The technical product is at least 99% pure and is formulated for use in wettable powders, dusts, granular products, flowable suspensions and baits (Von Rumker et al., 1974).

Products formulated for use range from 0.5% (aerosol) to 80% (wettable powder) concentrations of carbaryl.

3. Tolerances. Under Section 408 of the Federal Food, Drug, and Cosmetic Act, the Environmental Protection Agency determines legally permissible tolerances for pesticidal chemical residues in or on raw agricultural products. Established tolerances for carbaryl and its metabolites, including 1-naphthol, are listed in 40 CFR 180.169, and are summarized below:

i. 100 parts per million (ppm): alfalfa, alfalfa hay, barley (green fodder and straw), bean forage, bean hay, clover, clover hay, corn fodder, corn forage, cotton forage, cowpea forage, cowpea hay, grass, grass hay, oats (green fodder and straw), peanut hay, pea vines, rice straw, rye (green fodder and straw), sorghum forage, soybean forage, soybean hay, sugarbeet tops, wheat (green fodder and straw).

ii. 40 parts per million: almond hulls.

iii. 12 parts per million: blackberries, boysenberries, collards, dandelions, dewberries, garden beets (tops), kale, loganberries, mustard greens, parsley, raspberries, spinach, Swiss chard, turnips (tops).

iv. 10 parts per million: apples, apricots, asparagus, bananas, beans, blueberries, broccoli, brussels sprouts, cabbage, carrots, cauliflower, celery, cherries, Chinese cabbage, citrus fruits, cranberries, cucumbers, eggplants, endive (escarole), grapes, kohlrabi, lettuce, melons, nectarines, okra, olives, peaches, pears, peas (with pods), peppers, plums (fresh prunes), pumpkins, salsify (tops), sorghum grain, strawberries, summer squash, tomatoes, winter squash.

v. 5 parts per million: corn (kernels and kernels plus cob, determined after removing husks present when marketed), cottonseed, cowpeas, garden beets (roots), horseradish, meat and fat of poultry, parsnips, peanuts, radishes, rice, rutabagas, salsify (roots), soybeans, turnips (roots).

vi. 1 part per million: almonds, chestnuts, filberts (hazel nuts), pecans, and walnuts.

vii. 0.5 part per million: maple syrup.[1/]

viii. 0.2 part per million (negligible residue): potatoes.

ix. Zero: grains of barley, oats, rye, and wheat.

1/ In addition, an interim tolerance of 0.5 ppm has been set for residues of carbaryl in eggs (40 CFR 180.319).

C. Regulatory History

Carbaryl was first registered for use in 1959 as an insecticide on cotton. With subsequent registrations, the total number of approved sites of application has risen to more than 80. The first tolerances were granted in 1959, and the number of tolerances granted now numbers 101. The most recent tolerance (0.2 ppm on potatoes) was granted in 1975.

In November 1968, carbaryl was classified as highly toxic to bees subject either to direct exposure or to indirect exposure via residues. As a result, carbaryl products intended for foliar applications to crops and trees, and for mosquito abatement, were required to bear cautionary statements/warnings against use in the vicinity of bees.

D. Environmental Fate

1. Hydrolysis. Carbaryl is fairly stable to hydrolysis in acidic and neutral mediums. In an alkaline medium at ph 9 to 10, carbaryl decomposes to 1-naphthol and N-methyl carbamic acid. N-methyl carbamic acid is unstable, and it further decomposes to carbon dioxide and methyl amine (Christie, 1969; Ukeles, 1962).

2. Photoreactivity. Pure carbaryl is stable in daylight and in ultra-violet light. When it is irradiated in ethanol solution, its products include 1-naphthol and other, unidentified products which have anticholinesterase action (Crosby et al., 1965). 1-Naphthol has also been identified as a product which occurs when carbaryl is irradiated in aqueous solution (Aly, 1971).

3. Fate of Carbaryl in Soil—Persistence Studies. Carbaryl is moderately persistent in soil. LaFleur (1976) applied carbaryl to a Congaree sandy loam field plot at 25.4 kg/ha. After four months, carbaryl was not detected in the upper 20 cm of soil. After 16 months, 6% of the applied carbaryl was found in the upper one meter of soil. LaFleur (1976) also demonstrated that retention of carbaryl varies widely depending on the soil. Using columns of six different South Carolina soils, he found that those with high organic content were able to absorb several times as much carbaryl as sandy soils. Johnson and Stansbury (1965) applied carbaryl in three different concentrations and found it to have a "half life" of approximately eight days under normal conditions. After 40 days, Johnson and Stansbury did not detect any carbaryl.

Carbaryl is metabolized in the soil by several species of microbes (Bollag and Liu, 1971a, 1971b). Through hydrolysis, carbaryl is broken down into 1-naphthol and other, unidentified metabolites.

E. Biological Fate — Metabolism

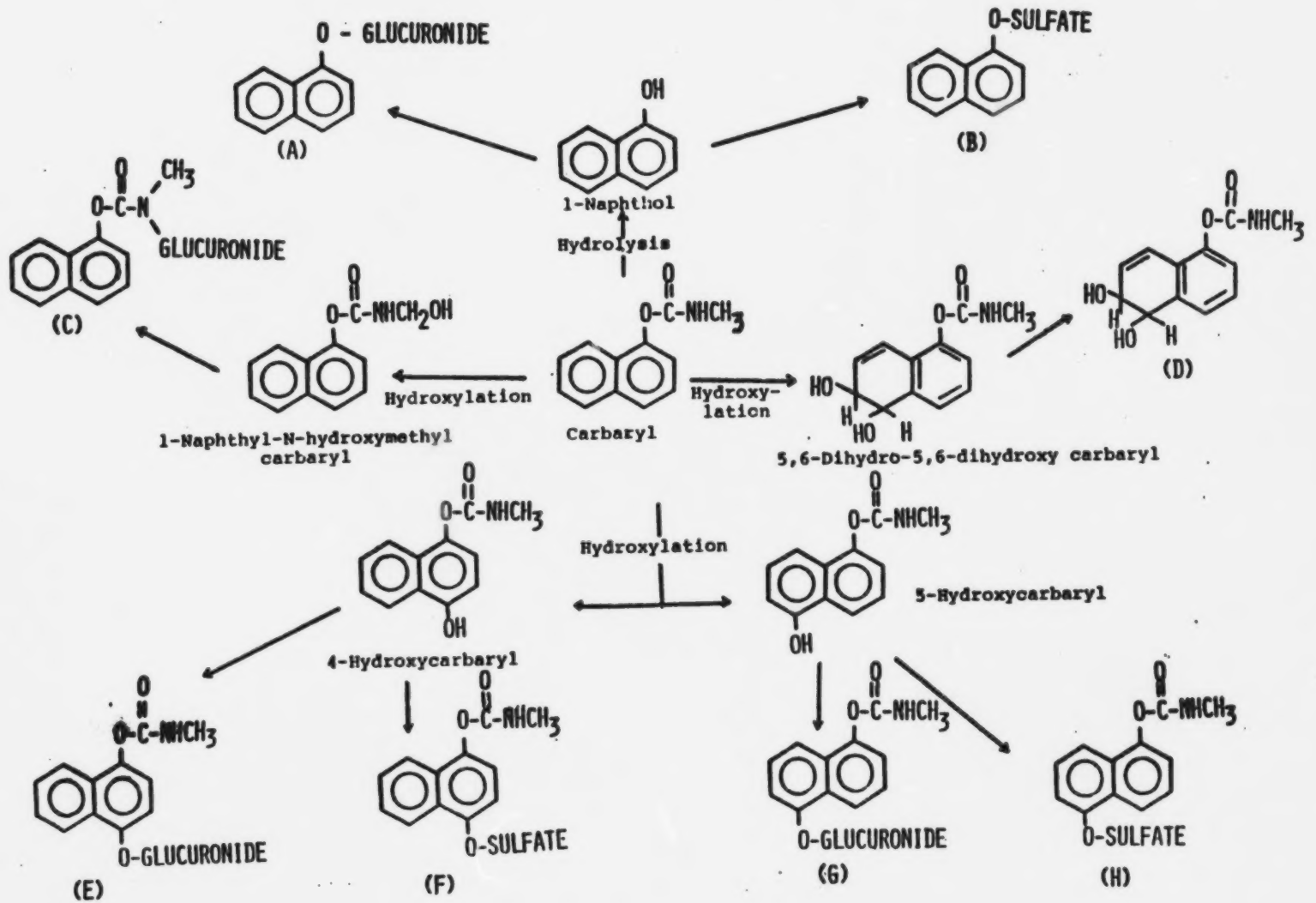
The metabolism of carbaryl has been studied in a variety of animals, plants and microbes (Declume and Bernard, 1977; Menzie, 1969). In general, the primary processes include hydroxylation, hydrolysis, and epoxidation to form numerous metabolites, with 1-naphthol being a major breakdown product. Major animal conjugates of 1-naphthol include water-soluble sulfates, glucuronides, and mercapturic acids. In plants, glycosides are formed.

Studies indicate that mammals metabolize carbaryl either by hydrolysis to form 1-naphthol or by any of four hydroxylation steps affecting the ring or the N-methyl group (Menzie, 1974). Whether carbaryl is initially broken down by hydrolysis or by hydroxylation, carbaryl metabolites are ultimately conjugated

by sulfation or glucuronidation, and the conjugates are eliminated in the urine and/or feces. In some cases, some of the hydrolysis and hydroxylation products have been detected in the urine at low levels in the free form (Menzie, 1974). For most mammals, hydroxylation and glucuronidation are the preferred mechanisms.[2/] The primary metabolites of carbaryl and their routes of formation are shown in Figure 1 as follows:

2/ Although most animals metabolize at least a portion of administered carbaryl through hydrolysis, this does not seem to be a significant mechanism in monkeys and pigs (Menzie, 1974).

FIGURE 1--PRIMARY CARBARYL METABOLITES IN MAMMALS AND ROUTES OF FORMATION (HYDROLYSIS OR HYDROXYLATION)



Various studies have been performed to elucidate the identity and quantity of carbaryl metabolites in various mammals, including man, the dog, and the rat. The studies to date indicate that the metabolic patterns observed in mammalian species are qualitatively identical, with few exceptions. The nature of the metabolites produced is fairly consistent, although there are specie variations in quantity and order of distribution, with some species producing metabolites not found in others (Kuhr et al., 1970; Baron et al., 1969; Dorough et al., 1964; Knaak et al., 1965; Krishna et al., 1966).

In man, the primary mechanism of carbaryl metabolism appears to be hydrolysis. As identified in non-radioactive studies by Knaak, the major urinary metabolites are 1-naphthyl glucuronide and 1-naphthyl sulfate. In addition, 4-hydroxy-carbaryl glucuronide has also been identified in human urine, indicating that humans metabolize carbaryl through the hydroxylation as well as the hydrolytic pathways.

The metabolic profile of the dog appears to be qualitatively similar to that of man and displays minor quantitative differences. In a 1967 study performed by Knaak and Sullivan in three dogs using radioactive carbaryl, carbaryl metabolites were found to be eliminated by both the urinary and fecal routes. The only identified urinary metabolite was the glucuronide of 5,6-dihydro-5,6-dihydroxy-carbaryl, a hydroxylation product. A second major metabolite, chromatographed as 1-naphthyl glucuronide, did not exhibit the fluorescent properties expected of this compound. The neutral metabolites appeared to include an unspecified naphthalenediol; other tentatively identified compounds were the 4-hydroxycarbaryl glucuronide and the 4-hydroxycarbaryl sulfate.

In a subsequent radio-labeling study performed by Sullivan et al. (1972a), metabolites identified in the dog and dog liver preparations included neutral compounds, which although not specifically identified, were believed to include carbaryl, 1-naphthol, and 4 and 5-hydroxycarbaryl compounds. Other compounds identified were 5,6-dihydro-5,6-dihydroxy-carbaryl, hydroxycarbaryl glucuronide, hydroxycarbaryl sulfate, and a non-fluorescent compound tentatively identified as 1-naphthyl glucuronide. The absence of fluorescence for the compound believed to be 1-naphthyl glucuronide may be due to the presence of an unknown metabolite with fluorescent-quenching properties co-chromatographed with the 1-naphthyl species. The identity of metabolites excreted in the feces was not investigated.

Studies in the rat have demonstrated that carbaryl is metabolized by both the oxidative and hydrolytic routes in the species. In a 1964 study performed by Dorough and Casida, carbaryl was incubated with rat liver microsomal preparation; the metabolites identified included 1-naphthol, 4-hydroxy-carbaryl, 5-hydroxycarbaryl and 1-naphthyl-N-hydroxy-methyl-carbamate. The study detected, but did not identify, four other major metabolites, two of which contained intact carbamate groups. A radio-labeling study performed in 1965 by Knaak et al. in male rats identified the sulfate and glucuronide conjugates of naphthol, 4-hydroxycarbaryl and 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide as the major urinary metabolites. In later studies of carbaryl (Matsumura and Ward, 1966; Strother, 1972), the metabolism in the intact rat liver confirmed the findings of the Dorough and Casida rat liver microsome study: the metabolites which were identified were 1-naphthyl, 4- and 5-hydroxy-carbaryl, and 1-naphthyl-N-hydroxymethylcarbamate. Another study of carbaryl metabolism in the rat identified the presence of thioether conjugates in rat bile and urine.

A recent preliminary study by Dorough (1979) regarding the comparative chemical nature of carbaryl metabolites in the rat and the dog indicated that there is no appreciable difference in the manner and rate at which carbaryl equivalents are voided from these two species.

In summary, although the comparative carbaryl metabolism in such species as man, dogs, and rats has not been fully detailed, there is evidence that all these species metabolize carbaryl by similar pathways and that the metabolites produced are qualitatively similar.

F. Pesticide Incident Monitoring System (PIMS) Reports

A review of the files of the Pesticide Incident Monitoring System (PIMS) revealed 441 carbaryl-related incidents (active ingredient 1-naphthyl methylcarbamate or 1-naphthyl N-methylcarbamate) reported for the period 1966 to January 1980. Of the 441 instances in which alleged adverse effects were reported, 258 involved carbaryl as a sole active ingredient, and 183 involved carbaryl in combination with other ingredients. One hundred ninety-three of those incidents which were linked to carbaryl as a sole active ingredient involved humans, and PIMS records describe these incidents in terms of 3 fatalities and 16 hospitalizations, 176 persons who received medical attention, 124 who were affected and not treated, and 3 who were involved but not affected. One hundred forty-four of those incidents which were linked to carbaryl in combination with other ingredients involved humans. PIMS records describe these incidents in terms of 2 fatalities and 35 hospitalizations, 91 persons who received medical attention, 18 who were affected and not treated, and 1 person who was involved but not affected.

No clear trend in the number of carbaryl-related incidents reported over ıe years emerges from PIMS records. Fewer than 10 incidents were recorded each year from 1966 through 1971 and again in 1979. Fewer than 50 incidents were reported in 1972 and in 1974. Between 50 and 100 incidents were recorded in 1973 and in 1975. One hundred or more incidents were recorded in 1976 and in 1978.

PIMS records indicate a wide range of sites and circumstances associated with carbaryl-related incidents. The largest category, however, comprised incidents in and around the home, and the second largest category comprised agricultural incidents.

Among home incidents, ingestion of the pesticide was the most common type of episodes involving children. Other types of home incidents included accidental splashing or spilling, contamination while spraying (home garden ge), and contamination via contact with residues on home garden crops. Agricultural incidents most commonly involved field workers laboring for a number of days in fields which had been sprayed or dusted with carbaryl or a combination of pesticides including carbaryl.

Two points should be emphasized in connection with the overview given above: 1) Recorded incidents include only those which have actually been reported to PIMS. Because other, unreported incidents have in all likelihood occurred, the number of incidents cited here should be considered a minimum. 2) Not all incidents recorded for the period 1966 to January 1980 have been formally confirmed as results of pesticide exposure (PIMS, 1980).

III. CARBARYL AS A POTENTIAL RPAR CANDIDATE

A. Introduction

Sec. 3(a) of FIFRA requires all pesticide products to be registered by the Administrator of EPA before they may be sold or distributed. Sec. 6(b) of FIFRA authorizes the Administrator to issue a notice of intent to cancel the registration of a pesticide or to change its classification if it appears that the pesticide or its labeling "does not comply with the provisions of [FIFRA] or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects on the environment." Thus the Administrator must cancel the registration of a pesticide whenever he or she determines that it no longer satisfies the standard for registration which requires, among other things, that the pesticide not cause "unreasonable adverse effects on the environment" [sec. 3(c)(5) of FIFRA]. These "unreasonable adverse effects" are defined in sec. 2(bb) of FIFRA to include "any unreasonable adverse effects to man or the environment, taking into account the economic, social and environmental costs and benefits of the use of any pesticide."

EPA has designed a public process to gather risk and benefit information about suspect pesticides so that the Administrator may make balanced decisions concerning them. The process is known as the Rebuttable Presumption Against Registration (RPAR) process; it is set out in 40 CFR 162.11. In broad summary, these regulations stipulate certain criteria of risk and provide that an RPAR arises if the Agency determines that any of these hazard criteria have been met. If it is determined that according to available scientific evidence, one or more of the criteria have been met, then the Agency issues an announcement

of "Rebuttable Presumption Against Registration." The 40 CFR 162.11 regulations require that an opportunity then be provided for registrants, applicants, and interested persons to submit evidence to rebut the presumption, or evidence relating to the economic, social, and environmental benefits of any use of the pesticide. If the presumptions of risk are not rebutted, the evidence on the benefits of the pesticide is evaluated and considered along with the information on the risks from the pesticide. The Agency then analyzes various methods of reducing the amount of risk from the pesticide together with their costs and determines whether the pesticide can be regulated so that the benefits of continued use outweigh the competing risks. If measures short of cancellation cannot reduce the risks associated with any given use of the pesticide to a level which is outweighed by benefits, the use in question must be cancelled.

Carbaryl was referred to the EPA as an active ingredient suspected of meeting 40 CFR 162.11 risk criteria warranting a rebuttable presumption. Teratogenicity as a potential hazard of the pesticide was the primary basis of concern, but carbaryl has also been subject to examination for other possible adverse effects. Hence the Agency initiated an intensive review of the presently available toxicological evidence. The following effects are at issue in this review: teratogenicity and fetotoxicity, mutagenicity, oncogenicity, neurotoxicity, and viral enhancement. The conclusions of this decision document constitute the Agency's determination as to whether a rebuttable presumption against pesticide products containing carbaryl is warranted at this time.

B. Teratogenic and Fetotoxic Effects

40 CFR 162.11 (a)(3)(ii)(B) provides that a rebuttable presumption against a pesticide's continued registration shall arise if that pesticide's ingredient(s) "produces any ... chronic or delayed toxic effect [other than an oncogenic or mutagenic effect] in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account an ample margin of safety." To determine whether or not carbaryl might pose a teratogenic or fetotoxic hazard warranting a rebuttable presumption, the Agency has conducted a thorough review and evaluation of the currently available literature.

By way of introduction to the following discussion of teratogenicity and fetotoxicity as possible adverse effects of exposure to carbaryl, it will be useful to address some basic issues of definition. Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring in utero. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) — or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while

the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic—fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to in utero exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up." Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting after effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/fetal effect in one species might, under a given agent, react in another species in a more serious and

irreversible manner. The Agency must therefore consider all such deviations from normal development in its risk assessment process, regardless of any appearance of reversibility.

1. Laboratory Data. Data concerning the potential of carbaryl to induce adverse prenatal effects in mammalian species are extensive, more extensive than has been the case for other pesticides which have come under Agency review. Numerous studies have been conducted on diverse mammalian species including the mouse, rat, gerbil, hamster, guinea pig, rabbit, swine, sheep, monkey, and dog. All of the studies are not of equal utility, however, for purposes of assessing the potential of carbaryl to act as a perinatal toxicant in the environment. A number of the studies were done with inappropriate protocols, and some of the older American studies, as well as many of the foreign papers, are seriously flawed by inadequate presentation of data. The Agency has therefore chosen to base its conclusions concerning the potential teratogenic and fetotoxic risk from carbaryl exposure primarily on those studies which are valid and interpretable (Chernoff, 1976 [updated 1980]). Because many of the studies discussed below are seriously flawed, detailed statistical analyses and "p" (pool) values are generally not included, since such detail would suggest a false statistical rigor. In the following discussion, an overall weight of evidence approach is applied in an analysis of currently available data.

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a. Rodents. A number of studies have been conducted on the teratogenic potential of carbaryl in various species of rodents. In the mouse, Murray et al. (1979) showed that administration of carbaryl during gestation by oral gavage (as much as 150 mg/kg/day) or in the diet (5660 ppm) did not result in increased incidences of terata (birth defects) even at doses producing maternal toxicity. Some reduced fetal weight and skeletal ossification were noted in the dietary experiment, but significantly reduced maternal weight gain was also noted at this dose level.[3/]

The potential of carbaryl to affect the development of the rat has been studied in a number of experiments. Weil et al. (1972) administered carbaryl in the diet and observed fetal toxicity only at the highest dose (500 mg/kg/day), which also produced significant reductions in maternal weight gain. At dose levels where no maternal effects were noted (100 mg/kg/day), there were no fetal effects. No terata were seen at any dose level.[4/]

3/ A number of other studies have been done on the mouse, but are of less importance for a variety of reasons. A study by Guthrie et al. (1971) attempted to determine the ability of mice to adapt to pesticide poisoning over a series of generations. The protocol was not comparable to any known teratological study, and therefore the results (no terata seen) are impossible to interpret.

The experiments done by Benson et al. (1967) used very small numbers of animals and low doses (30 mg/kg/day), and this study (which showed no effects) is therefore of little value in determining the fetotoxic potential of carbaryl. A series of studies was done at the Bionetics Laboratory (1968), but the inappropriate route of administration (subcutaneous) and a failure to replicate effects that were initially noted also render this set of studies of little regulatory value.

4/ A study by Hart et al. (1972) also used the rat to determine the teratogenic potential of carbaryl. The dose administered was low (37.5 mg/kg/day), and no effects were noted. There have also been a number of studies done by scientists in Russia. These lack critical details (number of animals tested, number of litters examined, source and purity of carbaryl) and are therefore extremely difficult or impossible to interpret.

Likewise the potential of carbaryl to affect the actual process of reproduction has also been studied in the rat. Weil et al. (1973) administered the compound either by gastric intubation, or in the diet. Maternal toxicity was evident as animals showed cholinesterase inhibition, decreased body weight, and increased mortality. A depression of the reproduction index occurred in the first generation of animals receiving 100 mg/kg/day by oral intubation. This depression did not occur in subsequent generations, however, and may have been a random occurrence since the chances of selecting a resistant sub-strain in one generation are minimal. Embryo or fetal lethality was seen in litters of this dose group by a decrease in viable fetuses in the F2a - F2b generations and a decrease in the number of live pups born in the F1a - F2a and F2a - F3a generations. The interpretation of these effects should take into account the significant maternal mortality in treated groups at all breeding periods. No reproductive or fetal effects were seen at lower dose levels (25 mg/kg/day and less). Carbaryl-induced effects were far less severe in animals receiving the pesticide in the diet (200 mg/kg/day). Decreased body weight was noted in animals during the initial portion of the experiment, and an increase in the average number of days from the first mating to the birth of the litter was seen only in the F1a - F2a generation. No fetal effects were noted in this experiment.[5/]

5/ Collins et al. (1971) also performed a 3-generation study in the rat. Lack of information concerning the status of the mothers and, in some cases, incorrect methods of calculating the results render this study difficult to interpret. One example of these flawed calculations is in the calculation of the average litter size. Average litter size was calculated by dividing the total number of progeny by the number of females exposed to mating. Since animals which did not produce litters were not examined for pregnancy, the presumption of 100% pregnancy inherent in this calculation is unjustified. An error is therefore introduced into these calculations since an animal which failed to conceive would carry the same weight in the calculation as an animals whose entire litter died *in utero*. These are not the same phenomena, of course, and a sterile or "unwilling" animal might skew the results. The authors did not report significant neonatal effects at the lowest dose group (123 mg/kg /day).

Effects of carbaryl on the developing guinea pig were studied by Weil et al. (1973). Carbaryl was administered either by oral gavage, or in the diet. The high (200 mg/kg/day) oral gavage dose resulted in a significant reduction of maternal weight gain. (Recorded differences in maternal weight gain were not adjusted, however, to take differences in litter size into account.) There was no reduction in fetal weight and no increase in fetal anomalies observed. The results of dietary administration of carbaryl (as high as 300 mg/kg/day) were also completely negative in terms of fetal effects. The carbaryl administered in the diet appeared to induce less severe maternal toxic effects than did carbaryl administered by oral gavage. Weil et al. concluded that the dietary administration of carbaryl also had no effect on guinea pig development.[6/]

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6 / A study by Robens (1968) showed that carbaryl is teratogenic when administered to guinea pigs at doses which are lethal to a significant portion of the maternal animals. With doses of 300 mg/kg/day given on multiple days during gestation (precise days not specified in paper), there were abnormalities present in 11 of the 46 fetuses. This dosing regimen resulted in 40% mortality in the treated dams. The lack of significant protocol information in the paper (precise days dosed, number of litters with affected fetuses) and the high degree of maternal mortality render this study extremely difficult to interpret in terms of the developmental toxic potential of carbaryl.

There have been two other rodent species in which the potential of carbaryl to induce adverse developmental effects has been studied. Collins et al. (1971) studied the effects of dietary administration of carbaryl for three generations in the gerbil. The same problems of interpretation alluded to in connection with the work of Collins et al. (1971) with the rat apply to their work with the gerbil. (Refer to note 5 above.) It does appear that at the doses administered in this experiment, carbaryl resulted in some perinatal toxicity as reflected in smaller litter sizes and poorer survival to day 4 in many of the treated groups. The lowest dose level administered was 200 mg/kg/day.

Robens (1968) also studied the effects of carbaryl in the hamster. All doses resulted in significant maternal toxicity. No fetal effects were noted in 125 mg/kg dose group, although considerable maternal toxicity was noted (diarrhea, salivation, and uncoordination). The small number of litters examined in this study (maximum of 8), coupled with the maternal toxicity, make the interpretation of these results difficult, although it does not appear that the fetal hamster is sensitive to carbaryl.

b. Other Mammalian Species. Carbaryl's potential to adversely effect mammalian development has also been studied in a variety of non-rodent species. The rabbit has been studied by Murray et al. (1979), who found that carbaryl was teratogenic at doses which induced maternal toxicity. Omphaloceles (fissures in the ventral body wall) were noted in litters of dams given 200 mg/kg/day by oral gavage in oil (this dose also resulted in reduced maternal weight gain and diarrhea). A single case of omphalocele was also noted at the 150 mg/kg/day dose group, a dose which also produced some maternal toxicity (diarrhea). The rabbit has also been studied by Robens (1968), who administered 200 mg/kg/day in a gelatin capsule and observed neither maternal nor fetal toxicity.[7/]

Two series of experiments have investigated the potential effects of carbaryl on the development of the Rhesus monkey. Dougherty et al. (1971) administered doses of carbaryl to monkeys beginning on the day sperm were found in the vaginal tract and continuing throughout gestation. Pregnancy was verified by a bioassay for monkey chorionic gonadotropin (MCG) using female mice. The control group comprised 5 pregnant females. The 2 mg/kg/day and 20 mg/kg/day groups consisted of 2 and 6 pregnant females, respectively. An increase in abortions was seen in the treated groups. Specifically, 2 abortions were reported in the 2 mg/kg/day group, 3 in the 20 mg/kg/day group,

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7 / Shaffer and Levy (1968) administered dietary doses of 10 and 30 mg/kg/day to the rabbit during gestation. These are low doses, and no effects in either the maternal or fetal animals were seen.

nd 1 in the control group. Of the five abortions noted in treated animals, all but one occurred early in pregnancy. Since fetuses were not recovered in these early abortions, and since early abortion in the Rhesus monkey is difficult to detect and may be confused with placental bleeding, the crucial factor in this first study would be the validity of the pregnancy test used. No terata were noted in any of the recovered fetuses or neonates. In a second study (Dougherty et al., 1974), Rhesus monkeys were given carbaryl on days 20-38 of gestation after verification of pregnancy. Pregnancy was determined by radio-immunoassay for HCG, a different method from that used in the previous study (Dougherty et al., 1971). These experiments did not indicate any carbaryl-related abortifacient (abortion-inducing) effect. No terata were seen in any of the groups. Two infants in the 20 mg group had low birth weights, one of which subsequently died at 10 days of age. The growth of infants of carbaryl-treated mothers was not different from the growth of control infants.

These two studies (Dougherty et al., 1971, and Dougherty et al., 1974) are not comparable in two crucial respects. The first is the validity of the respective pregnancy tests used in these studies since the key finding in the first study involved early abortions, which are difficult to detect. The second, and more important, is that the dosage regimen differed markedly in the two studies, the first being throughout gestation, while the second was during the period of major organogenesis. Thus animals in the first study received carbaryl during the first part of pregnancy when early abortions might be more readily induced, while in the second study the compound was administered only during the period of organogenesis, when pregnancy is further advanced. Also, the cumulative dosage differed, and at day 38 of pregnancy, animals in the first study had received approximately 27 doses (5 times per week), while animals in the second had received 19 doses. The second study therefore does

not negate the first, which may have indicated an early abortifacient potential or carbaryl. The very small number of animals used in the first study (a total of 13 comprised controls and two treatment groups), coupled with the difficulty in accurately identifying early abortion by the method employed, allows no definite conclusions to be made. An additional study of the potential of carbaryl to induce early abortion in the monkey would be necessary to answer this question raised by the first study (Dougherty et al., 1971). It should be noted that neither study in the monkey indicated any teratogenic potential of carbaryl in that species.

Two studies have attempted to determine the teratogenic potential of carbaryl in the beagle dog. Smalley et al. (1968) administered the pesticide in the diet throughout gestation at doses ranging from 3.125 mg/kg/day to 50 mg/kg/day. Birth defects were found in dose groups of 6.3 mg/kg/day and above. The defects were of a broad spectrum and included lack of tail, agenesis of external genitalia, failure of the pubis and ischium to develop, openings in the ventral body wall, and the visceral agenesis. Teratogenic effects were seen in 11.6% of the pups born to dams receiving carbaryl as compared to 0% in the controls. "Difficult births" (dystocia) were seen in all treatment groups and were absent in the controls.

Imming et al. (1969) attempted to repeat the Smalley study in the dog. They administered carbaryl throughout gestation at doses ranging from 2.0 to 12.5 mg/kg/day in the diet. They found a small increase in still births in the 12.5 mg/kg/day and 5.0 mg/kg/day groups, as well a slight reduction in survival until weaning at the highest dose level. Defects were seen in the 5.0 and 12.5 mg/kg/day dose groups and included umbilical hernia, cleft palate, and gastrointestinal anomalies. No defects were seen in the low dose (2.0 mg/kg/day) or control groups. The authors also reported that some of the bitches had difficulty during parturition. It would therefore appear that both

the pregnant female and fetal weight are sensitive to carbaryl and that this compound is teratogenic in this species at doses of 5.0 mg/kg/day or greater. The Agency is considering whether the dog studies should be repeated, with special attention paid to sufficient numbers of animals in the dose groups, the condition of the bitches throughout the period of dosing, and possibly maternal and fetal blood levels of the compound.[6/]

After reviewing all the above data, two general conclusions may be drawn concerning the potential of carbaryl to affect mammalian development. The first is that the administration of carbaryl to pregnant animals (at sufficiently high dose levels and/or sufficient duration of treatment) may result in adverse effects to the embryo or fetus. Of those studies from which definite conclusions may be drawn, carbaryl has been shown to produce teratogenicity in the guinea pig, rabbit, and dog; and fetotoxicity in the mouse, rat, and gerbil. The second conclusion which may be arrived at is that these effects

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6/ Two additional non-rodent species have been used in carbaryl studies. Earl et al. (1973) reported on experiments in which carbaryl was administered in the diet to swine throughout gestation. Fetal resorptions and stillborn were 22% in the 3 mg dose, 21% in the 15 mg dose, and 3% in controls in the first of the experiments. In the second experiment, resorptions were not noted, but the rate of stillborns was not dose-related, being 2% in the controls, 11% in the 15 mg dose, and 2% in the 32 mg dose. There was no evidence of any dose-related fetal anomalies in either study. No information is given concerning the effects of carbaryl on the dams. The swine studies are perhaps suggestive of some fetal toxicity, but there is not sufficient information at present to make any definite conclusions concerning the effects of carbaryl on this species.

Pindyck (1967) studied the effects of carbaryl on pregnant sheep. Two cardiac anomalies were found in the treated groups, but the small numbers of pregnant animals, together with no knowledge of the normal incidence of such anomalies, make it impossible to draw definite conclusions concerning this study.

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ave generally occurred only at dose levels which are grossly toxic to the maternal animal. Adverse developmental effects have been seen at levels which resulted in maternal death in the guinea pig, cholinergic toxicity in the rabbit, and weight loss in the rat and mouse (the health status of the maternal gerbils was not given in the published study concerning that species). The dog appears to be the only exception to this conclusion, and in this species the treated females had difficulty giving birth, a possible sign of carbaryl-induced maternal toxicity.

The Agency fully acknowledges that the evaluation of these studies in terms of their applicability to the human population must be done with great care. In assessing the totality of the experiments, the Agency recognizes that carbaryl has been tested in an extremely wide variety of species and has been found to be teratogenic only in three species (guinea pig, rabbit, and dog), of which defects were found in only one species (the dog) at doses below those causing maternal toxicity. In view of the circumstance that there are adequate prenatal studies in eight species, it would appear that carbaryl is not a potent teratogen. This same close dose relationship exists between maternal toxicity and forms of adverse fetal effects other than teratogenicity.

In judging the relevance of embryonic fetal effects which are "confounded" by gross maternal toxicity, several factors must be kept in mind. In an experiment where significant differences are seen between control and treated groups, these differences are by definition attributable to treatment. If adverse perinatal effects are found in carbaryl-treated litters, the cause of

these effects is understood to be carbaryl. Even if these effects are seen only in litters of mothers who were themselves adversely affected by the carbaryl treatment, we must still conclude that carbaryl is the cause of the developmental effects.

When the Agency begins to consider the relevance of such studies to the human population, however, other factors must be considered. Foremost are possible mechanisms by which the effect in the developing organism was obtained. If, for instance, a compound is administered which affects the maternal animal's desire to eat, the mother may lose weight and be unable to provide sufficient nourishment to either the fetal or neonatal animal. A study such as this raises the question, however, whether any type of food restriction would have resulted in the same effect. If this is the case, the primary effect would be the food restriction, and the mechanism for producing it would become almost incidental. In the study by Robens (1968), for example, carbaryl produced terata in guinea pigs at doses which resulted in 40% maternal mortality. While carbaryl was by definition the teratogenic agent, it is very possible that the actual cause of the terata lies in some part of the general debilitation of the surviving animals. Without a control group which is equally sick it is impossible, however, to distinguish between the unique effects of carbaryl and the effects of the severe maternal toxicity. In all the species tested, with the exception of the dog, adverse fetal effects were not seen when there was no maternal toxicity. There is a valid question, therefore, as to whether carbaryl itself has any properties which render it fetotoxic or whether carbaryl, like most pesticides, can cause severe maternal toxicity when administered at sufficiently high doses, and some aspect(s) of this toxicity in turn results in adverse developmental effects.

Another aspect of the relationship of maternal toxicity to adverse fetal effects is the relationship between the effect doses of both types of effects. It is generally felt that a compound which is fetotoxic at much lower dose levels than it is maternotoxic has the potential to be a greater human hazard than one in which the effect levels are similar. The reason for this is that the former case is far more insidious environmentally, since a slight rise in terata or other fetal toxicity is extremely difficult to identify by epidemiological means. Acute health problems in the adult population are easier to identify, and corrective actions may be swiftly taken. The relationship between maternal and fetal toxic dose levels clearly approaches equality in most species tested with carbaryl. Even in the case of the dog, some signs of maternal toxicity occurred at doses where terata were noted, indicating that in this species, too, adverse developmental effects were not seen at doses below those which elicited maternal toxicity.

In view of the overall weight of evidence of studies which are valid and interpretable, the Agency has concluded that currently available data on carbaryl do not indicate that a rebuttable presumption on the basis of teratogenic and fetotoxic effects is warranted at this time. In the Agency's judgment, the extremely high doses of carbaryl used to elicit effects in the developing organism, coupled with the positive correlation of maternal and fetal toxicity in the multiple species tested (the dog being a possible exception), indicate that carbaryl would not constitute a potential human teratogenic or reproductive hazard under proper environmental usage. As articulated above, however, the Agency is considering whether the dog study should be repeated, with special attention paid to sufficient numbers of animals in the dose groups, the condition of the animals throughout the period of dosing, and possibly maternal and fetal blood levels of the compound (Chernoff, 1977 (updated 1980)).

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2. New Jersey Epidemiological Studies to Assess Carbaryl-Related Birth Defects. The State of New Jersey, Department of Health, carried out epidemiological studies for adverse reproductive effects and birth defects due to carbaryl spraying for gypsy moths (Halpin, 1980). Three counties were selected for the studies: Cape May, Morris, and Monmouth. These counties were selected because residents and the press alleged that human and animal abortions and birth defects occurred because of carbaryl spray exposure.

The survey was conducted by the staff of Parental and Child Health Services of the New Jersey State Health Department to investigate several cases of birth defects that occurred in Cape May County. Five cases of birth defects were reported from births during the period from December, 1979, to January, 1980. The coincidence of these cases with the spraying of carbaryl in the spring of 1979 led to speculation that carbaryl was the cause of the defects. The study was carried out to explore the possibility of a correlation between use to control gypsy moths and the occurrence of the birth defects. In particular, the study was initiated to answer two questions: 1) Is there an increased rate of birth defects in municipalities where carbaryl is used in aerial spraying for gypsy moths? and 2) Is there a temporal correlation between the occurrence of birth defects and this spraying?

Live births (34,355) from January 1, 1977, through March 31, 1980, were surveyed in Morris, Monmouth, and Cape May counties, which were chosen on the basis of their level of spraying and their common suburban/rural characteristics.

The records of all ten hospitals with maternity services in these counties were reviewed by the Health Department. Three physicians studied and classified the possible cases, scoring only major birth defects (Down's Syndrome, major organ system defects, cranio-facial defects, defects of nervous

system development, and absence of any limb or portion of a limb). Minor birth defects (webbing between fingers, extra digits, minor abnormalities of the external genitalia, and maldevelopment of the feet) were not included in the statistical analysis, nor were birth marks/discolorations, hip joint dislocation, malposition of the feet, hernias, and undescended testicles.

The rate of birth defects per 10,000 live births was calculated for each municipality. These rates were then compared between those municipalities that sprayed carbaryl for gypsy moths and those that did not. Analysis of the possible temporal relationship between the spraying and the defects was based on a calculated two-week interval of probable conception.

For sprayed municipalities the average rate of birth defects was 44.36/10,000 live births, with a standard deviation of 64.44. The average rate for non-sprayed municipalities was 68.55/10,000 live births, with a standard deviation of 219.02. The State Health Department concluded that there is no difference between the rates of birth defects in sprayed municipalities and in non-sprayed municipalities.

Analysis of the time of conception and the spraying of carbaryl (no statistics given) also led to the conclusion that there is no difference between the sprayed and non-sprayed municipalities (Halpin, 1980).

C. Mutagenicity [9/]

40 CFR 162.11(a)(3)(ii) (A) provides that "a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) ... induces mutagenic effects, as determined by multitest evidence." 40 CFR 162.3(y) defines mutagenicity as "the property of a substance or mixture of substances to induce changes in the genetic complement of either somatic or germinal tissue in subsequent generations." Mutagenic chemicals are recognized as posing a potential risk to human health because of their ability to cause heritable changes in genes and chromosomes. Such germline changes can, for example, lead to birth defects or to the accumulation of deleterious mutations in the human gene pool. In addition, somatic mutations may be involved in the etiology of cancer.

To determine whether or not carbaryl might pose a mutagenic risk, the Agency has reviewed the currently available data and conducted an assessment of the mutagenic potential of carbaryl (RE&G, 1988). Specifically, the primary objective of a mutagenicity risk assessment is to determine the potential of a chemical to cause heritable germline effects in man, as the Agency has articulated in its Proposed Guidelines for Mutagenicity Risk Assessments, (Fed. Reg. 45(221): 74984-74988 (November 13, 1980)).

9/ Only those data which are pertinent to mutagenicity as a possible adverse effect of carbaryl per se are evaluated here. The possibility of a mutagenic risk posed by formation of N-nitrosocarbaryl in stomach physiology will be addressed in a separate Agency review of nitroso compounds. (See Appendix A for a summary of current information concerning the mutagenicity of N-nitrosocarbaryl.)

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In broad summary, carbaryl has been reported to produce gene mutations in bacteria, Drosophila, and mammalian cells in culture. However, there are several inadequacies in these studies. In addition, the results of cytogenetic tests imply that carbaryl may induce chromosomal effects in mammalian cells in culture, in whole mammals, and in plants, and carbaryl has been shown to cause primary DNA damage in cultured human cells. Collectively, all of these factors strongly suggest that carbaryl may act as a mutagen. To cause heritable effects in man, however, a chemical with intrinsic mutagenicity must reach the germinal tissue. Evidence that carbaryl and/or its active metabolites reaches the gonads is only suggestive. Adverse gonadal effects — e.g., abnormal sperm morphology, reduction in the number of spermatogonia and spermatozoa in the seminiferous tubules, and reduced sperm motility — have been observed in rodents exposed to carbaryl (Degraeve et al., 1976; Shtenberg and Rybakova, 1969; Kitagawa et al., 1977). In addition, abnormal sperm head morphology has been reported in workers with known exposure to carbaryl. Therefore, given the weight of evidence, carbaryl may have the potential to act as a germ cell mutagen. It should be emphasized, however, that carbaryl is not intrinsically a potent mutagen in the reported studies, and probably acts as a weak mutagen only. A review of the most pertinent evidence follows.

1. Evidence Concerning Point (Gene) Mutations. Point (gene) mutations are alterations which affect single genes. They are defined as intralocus changes (base-pair substitutions, frameshift mutations, small deletions, and additions).

a. Bacteria. Studies concerning point mutations in bacteria are summarized in Table 1 as follows:

TABLE 1 — POINT MUTATIONS IN BACTERIA

Reference	Test System	Strains	Activation	Concentration	Results	Comments
Ashwood-Smith et al., 1972	Reversion to tryptophan prototrophy in *E. coli*: spot test	WP2	None	10% solution in phosphate buffer saline (1mg/disk)	Negative	1. Data not presented. 2. Concentration of carbaryl is above solubility in water (0.01%; Carpenter et al., 1976).
Blevins et al., 1977	*Salmonella* test: plate incorporation and spot test	TA 1535 TA 1537 TA 1538 TA 98 TA 100	None	50 nanomole or 11.5 ug/plate dissolved in ethanol	Negative	1. Data not presented. 2. Spontaneous frequencies not given. 3. No positive controls for tester strains.
Cook et al. (abstract, 1977)	*Salmonella* test: plate incorporation	TA 100	None	0.2, 2, 20, 400 ug/plate	Weakly mutagenic at 400 ug/plate	1. Abstract without data. 2. Data obtained by personal communication (Cook, 1980).
DeGiovanni-Donnelly et al., 1968	Back mutation at indole locus of *Bacillus subtilis*	1681-	None	0.07% solution	Negative	1. Article was brief on methodology. 2. No positive controls.
DeLorenzo et al., 1978	*Salmonella* test: plate incorporation	TA 1535 TA 1537 TA 1538 TA 98 TA 100	Phenobarbital induced rats, S-9 liver preparation	10 ug to 1500 ug/plate, dissolved in DMSO (96-99% purity)	Negative with or without metabolic activation	Data not presented.

TABLE 1 — POINT MUTATIONS IN BACTERIA (CONT'D)

Reference	Test System	Strains	Activation	Concentration	Results	Comments
Egert and Greim, 1976	Mutation to prototrophy in *E. coli*: spot test	K12_ gal_ nad_ arg_ MTR	Mouse-liver microsomes and NADPH		Negative with or without activation	1. No positive controls. 2. Insufficient information on procedure employed. 3. Quantification of results not presented.
Egert and Greim, 1976	*Salmonella* test: liquid suspension	TA 1538	Mouse-liver microsomes and NADPH	186 uM	Increase in mutagenicity after activation	1. The positive results were not confirmed by repeating the experiment. 2. Dose related response not determined.
Elespuru et al., 1974	Forward mutation to novobiocin resistance in *Haemophilus influenzae*	Wild-type	None	0.1 mM	Negative	Did not examine a range of concentrations.
Fahrig, 1974	Spot test: back mutation to prototrophy in two auxotrophic strains of *Serratia marcescens*; to galactose prototrophy in *E. coli*				Negative	1. Report was a review of published and unpublished data. 2. Method and quantification of results not given.

TABLE 1 -- POINT MUTATIONS IN BACTERIA (CONT'D)

Reference	Test System	Strains	Activation	Concentration	Results	Comments
Fahrig, 1974	Liquid holding test: forward mutation to streptomycin resistance in *E. coli*				Negative	1. Report was a review of published and unpublished data. 2. Method and quantification of results not given.
Picsor and Nil Lo Piccolo, 1972	Reversion to prototrophy in *E. coli*: spot test	lac⁻ leu⁻ cys⁻	None	Commercial spray with 5% carbaryl	Negative	Method and quantification of results not presented.
McCann et al., 1975	*Salmonella* test: plate incorporation	TA 1535 TA 1536 TA 100 TA 98	Aroclor 1254 induced rats, S-9 liver preparation	10 ug up to 2000 ug/plate	Negative with or without activation	1. Mass screening of 300 chemicals. 2. Data presented only for 2000 ug/plate.
Marshall et al., 1976	*Salmonella* test: plate incorporation	TA 1535 TA 1536 TA 1537 TA 1538	Phenobarbital induced rats, S-9 liver preparation	50 ug to 1000 ug/plate, dissolved in DMSO	Negative with or without metabolic activation	1. Data presented only for 1000 ug/plate or where a 2-fold increase above background was observed. 2. Positive control data not presented.

TABLE 1 -- POINT MUTATIONS IN BACTERIA (CONT'D)

Reference	Test System	Strains	Activation	Concentration	Results	Comments
Nagy et al., 1975	Reversion to tryptophan prototrophy in *E. coli*: spot test	WP2 uvrA WP2	None	"Sevin" 85 WP (85% Carbaryl)	Negative	1. Data not presented. 2. Article not clear on dose of carbaryl used.
Rashid, 1978	*Salmonella* test: plate incorporation	Ta 1535 TA 1537 TA 1538 TA 100 TA 98	Aroclor 1254 induced rats, S-9 liver preparation	5, 25, 25, 325, 625 ug/plate without activation; 5, 10, 50, 250, 1250. ug/plate with activation	Weakly mutagenic in strain TA 1535	
Shirasu et al., 1976	*Salmonella* test: plate incorporation	TA 1535 TA 1536 TA 1537 TA 1538	None	0.02 ml of 1 mg/ml solution	Negative	1. Data not presented. 2. Insufficient information on method.
Shirasu et al., 1976	Reversion to tryptophan prototrophy in *E. coli*: spot test	WP2 uvrA WP2	None	0.02 ml of 1 mg/ml solution	Negative	1. Data not presented. 2. Insufficient information on method.
Uchiyama et al., 1975	Reversion to tryptophan prototrophy in *E. coli*: spot test	WP2	None	up to 10 mg/ plate	Negative	Data not presented

Originally, McCann et al. (1975) classified carbaryl as nonmutagenic in the Salmonella/microsome assay using four histidine-requiring strains. Metabolic activation did not augment the mutation frequency. Later, however, McCann and associates (personal communication, 1980) re-examined their data and conducted additional experiments at different concentrations of carbaryl to detect reversion at the histidine locus of Salmonella typhimurium TA 1535 (base-pair substitution sensitive strain). In the absence of metabolic activation they found that carbaryl appears to be weakly mutagenic. In one experiment, eight doses were examined (0 to 1000 ug/plate), and carbaryl exhibited a dose-response effect. At the carbaryl concentration 500 ug/plate, which yielded the maximum mutagenic response, the mutation frequency was increased to about 2-fold over control values.

The mutagenicity of carbaryl was also evaluated by Rashid (1978), employing five strains of Salmonella typhimurium. Rashid found that carbaryl produced a weak response in only strain TA 1535 (missense) at 125 ug/plate (1.6-fold increase above the spontaneous mutation frequency) and at 625 ug/plate (1.9-fold increase above the spontaneous mutation frequency) in the absence of metabolic activation. The presence of rat liver (S-9) microsomal enzymes did not increase the reversion frequency. Cook (abstract published in 1977) observed weak mutagenic activity (2-fold increase) on another base-pair substitution sensitive strain TA 100 at 400 ug/plate in the absence of metabolic activation (Cook, personal communication, 1980). Egert and Graim (1976) investigated the mutagenic activity of carbaryl using the Salmonella typhimurium strain sensitive to frameshift mutagens, TA 1538. After preincubation in mouse-liver microsomes and NADPH, 22 revertants/10^8 survivors were observed at 100 uM, whereas "<1" revertant/10^8 survivors were found in the nontreated control.

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In contrast, several investigators have reported negative responses with carbaryl using the Salmonella/Ames test. Other bacterial tests in which carbaryl has been reported as negative include back mutations in Bacillus subtilis, forward mutations in Haemophilus influenzae, and back and forward mutations in Escherichia coli (Table 1). It should be acknowledged that these negative results might be due to the relative sensitivity of the tests, solubility problems, or cytotoxicity.

b. Mammalian Cells in Culture. Ahmed et al. (1977) found approximately a 9-fold increase in the number of ouabain-resistant (OUA^R) mutants over spontaneous mutants after V79 Chinese hamster cells were treated in monolayer with 10 uM of carbaryl (66% cell survival). These CUA^R mutants were reported to be phenotypically stable. The apparent ability of carbaryl to generate ouabain-resistant mutants would be consistent with its activity as a base-pair substitution mutagen. Although carbaryl was found to be weakly mutagenic in this gene mutation assay, several inadequacies are apparent in this report which reduce the weight of the positive result: e.g., 1) a concentration-related increase in mutation frequency was not demonstrated, 2) concurrent positive controls were omitted, and 3) data were not presented to support the statement of phenotypic stability of the ouabain-resistant phenotype.

c. Drosophila: Sex-linked Recessive Lethal Test. Brezheskii (1972) exposed Drosophila melanogaster males to a 1% suspension of "Sevin" (85% carbaryl) in dilute sugar for 24 hours (50% survival). Brezheskii found a small increase ($p \leq 0.01$) in the percentage of complete (heritable mutations) and partial (not all mutations transmitted to progeny) recessive lethals (0.02%) as compared to control values (0.0% experimental control; 0.05 + 0.02% in

historical controls). If only the complete lethals are considered, the frequency observed (0.091%) is not significantly different from the spontaneous value. However, the Agency points out that a larger sample size would have to be examined to classify carbaryl either as weakly positive or as negative.

2. Evidence Concerning Primary DNA Damage. DNA repair tests do not measure mutation per se, but DNA damage as induced by chemical treatment of a cell. Microbial test systems measure this damage as cell killing. Mammalian cell systems -- both *in vitro* and *in vivo* -- measure the damage to DNA either directly, or as it is being repaired (DHEW, 1977).

Mammalian cells synthesize DNA during only one stage of the cell cycle. This is referred to as "scheduled DNA synthesis." However, when the cellular DNA is damaged, repair synthesis can occur when scheduled DNA synthesis is not taking place. This "unscheduled DNA synthesis" can be measured in synchronously growing cells as uptake of radioactive thymidine in the cell's DNA. It can be measured in cells in culture, or in meiotic and post-meiotic mouse spermatocytes *in vitro* (DHEW, 1977).

Ahmed et al. (1977b) have shown that exposure of virally transformed human cells (VA-4) in culture to carbaryl initiates unscheduled DNA synthesis at exposure as low as 1 uM as determined 1) by autoradiography and 2) by photolysis of bromodeoxyuridine (BrdUrd), which is incorporated into DNA during DNA repair synthesis. Metabolic activation by liver microsomes did not enhance carbaryl's ability to induce unscheduled DNA synthesis. The cytotoxicity of the carbaryl doses used was not given in this report.

Regan et al. (1976) treated a culture of human skin cells with [illegible]0 uM of carbaryl for 1 hour and found no evidence of DNA damage. The technique employed, however, was not the same as that used by Ahmed et al. (1977b). Regan et al. (1976) determined the sedimentation profiles in alkaline sucrose

gradients of cellular DNA treated with carbaryl as a detection method for DNA damage. This method of detection, however, may not be as sensitive as the (BrdUrd) photolysis method.

Siebert and Eisenbrand (1974) used a diploid strain of Saccharomyces cerevisiae D4 heteroallelic at the gene loci ade-2 and try-5 to assay for the ability of carbaryl to induce mitotic gene conversion in these loci. This is another assay that detects damage to DNA. In this organism, genetic activity (genetic damage) was not produced by a 16-hour carbaryl (1000 ppm) treatment. Yeast cells cultured in this solution showed only a 22% lethality.

3. Evidence Concerning Chromosome Effects. Mutagenesis involves not only point mutations but also the gain, loss, or rearrangement of portions of chromosomes as well as gain or loss of intact chromosomes.

Several cytogenetic studies have shown that carbaryl can cause chromosome abnormalities (colchicine mitosis, chromosome lagging, chromosome fragmentation, multipolar anaphases, anaphase bridges, multinucleated cells) in both meiotic and mitotic chromosomes of plants (Wuu and Grant, 1966; Amer, 1965; Amer et al., 1971; Amer and Farah, 1968; Brankovan, 1972). Although carbaryl is capable of breaking chromosomes in plants, predominantly it causes mitotic disturbances by interfering with the spindle mechanism. This may result in chromosome loss and/or gain. Russian studies provide suggestive evidence that carbaryl may also act as an antimitotic agent in human cells in culture and in rats (Shpirt, 1975; Kazarnovskaya and Vasilos, 1977; Vasilos et al., 1972, 1975).

In addition, Ishidate and Odashima (1977) studied the effects of carbaryl on chromosomes of cultured Chinese hamster fibroblasts. Three different doses (0.0075, 0.015, 0.03 mg/ml) were added to cell cultures (Ishidate, personal communication, 1980). At the maximum effective dose, 0.03 mg/ml (50% growth

inhibition dose), several types of chromosome aberrations (35% aberrant cells) were reported 48 hours after treatment. Specifically, chromatid gaps and breaks, chromosome breaks, translocation, ring formation, and fragmentation were observed with a higher frequency than in non-treated control cultures (1% aberrant cells). Although the authors stated the "gaps" were the predominant chromosomal effect, the frequency of occurrence for each particular type of aberration and the frequency of aberrations within a cell were not given. At lower doses, 0.015 mg/ml resulted in 24% aberrant cells, and 0.0075 did not appreciably affect chromosome structure (1% aberrant cells). The authors did not give the toxicity of these doses.

The dominant lethal assay in rodents, which detects chromosome damage in germ cells, was used both by Epstein et al. (1972) and by Weil et al. (1973). Using male mice, Epstein et al. (1972) administered 1000 mg/kg and 50 mg/kg (subtoxic doses) of carbaryl by gavage in daily portions over five consecutive days. Reportedly, this dosage schedule did not produce significant early fetal deaths or preimplementation losses. However, data were not presented in this report to support this statement. Weil et al. (1973) looked for dominant lethality in rats using a 3-generation study and found no significant lethal effects. The authors do not state if the carbaryl dosage level was the maximum tolerated dose. Furthermore, the number of males treated, the number of virgin untreated females mated with each treated male, and the number of implant and fetal deaths per female of test or control groups are not given in this report. It should be noted that in general the dominant lethal assay is recognized as an insensitive test for purposes of detecting weak mutagens. This is because of the small number of animals used in such a study and because of the high background of fetal wastage observed in control animals. In addition, chromosomal effects are usually observed at higher chemical doses than are point (gene) mutations (REAG, 1980).

4. Evidence Concerning Whether Carbaryl Reaches the Germinal Tissue. In order for any mutagen to cause genetic alterations that may be inherited by future generations, it must reach the gonads. As the Agency articulated in its Proposed Guidelines for Mutagenicity Risk Assessments, "Evidence that a chemical reaches or affects the germinal tissue, as provided by such sources as data demonstrating the alkylation of DNA or other cellular macromolecules, unscheduled DNA synthesis, sister chromatid exchange, or chromosome aberrations, in germinal cells; and non-specific accumulation of radioactive label in the gonads following administration of the labeled chemical [is considered a factor which contributes insight into the mutagenic activity of a chemical substance]. Other relevant evidence includes adverse gonadal effects following acute, subchronic or chronic toxicity testing; and adverse reproductive effects, such as decreased fertilization index, reduced sperm count, or abnormal sperm morphology " (Fed. Reg. 45[221]:74997). Numerous inadequacies are apparent in the available reports concerning the adverse gonadal effects which might be associated with exposure to carbaryl, and the evidence reviewed below is considered suggestive rather than conclusive (REAG, 1986; Chaisson, 1988).

a. Epidemiological Data. The sperm-abnormality assay is an indicator that a chemical agent may be damaging the germ cells (Wyrobek and Bruce, 1978). Wyrobek et al. (1983) analyzed semen samples from 50 carbaryl production workers who had spent at least one year on the job. Thirty-four new hires provided semen for control purposes. Carbaryl-exposed males had sperm counts or sperm with fluorescent bodies (thought to be caused by meiotic nondisjunction) similar to control values. There was, however, a significant elevation ($p<.005$) of sperm abnormalities (abnormal head morphology) in currently exposed workers (52.5 $\pm$ 2.6%) as compared to controls (41.9 $\pm$ 2.1%).

Previously exposed workers (an average of 4.5 years since last carbaryl exposure) did not exhibit a significant elevation of sperm abnormalities from control values. Because of the small sample size, however, it cannot be established if carbaryl effects are reversible. When current workers were classified as low (e.g., supervisors, foremen, maintenance personnel) and high (baggers, operators) exposure groups, both groups were shown to have significant differences in sperm abnormalities from the control group. However, there were not appreciable differences between the high and low exposure groups. In addition, there was a negative correlation between the number of years exposed to carbaryl and the percentage of abnormal sperm in current workers. Wyrobek and coworkers speculated on several mechanisms to explain this odd relationship 1) the likelihood that men working longer may be exposed less because of seniority, 2) biological or pharmacological adaption to exposure, 3) selection for non-affected males. Although this study demonstrates a correlation between working in a carbaryl-exposed area and an alteration of human spermatozoa, this is suggestive evidence only that these effects are due to carbaryl and/or its metabolites. It must be established that these defects of sperm morphology are not the result of other factors, such as exposure to chemicals other than carbaryl.

In an earlier epidemiological study performed with workers at the same plant studied by Wyrobek et al. (1980), Whorton and Milby (1978) examined semen samples provided by 47 workers who volunteered to participate in the experiment.[19/] Thirty-six of these workers also provided blood samples for hormone assays. The mean age of the 47 volunteers was not given. However, Whorton and Milby's cohort of 99 workers (selected from employment records), which included the 47 contributors, was described as follows: 53 baggers, (mean age = 41.1 years), 23 operators (mean age = 34.3 years), and 23 others (mean age 44.8 years). The experiment included no onsite controls. However, the results of the semen counts were compared to semen counts obtained from 98 members of a composite control population (chemical plant workers not exposed to any known infertility-producing agents), the data used for control purposes having been provided by Environmental Health Associates, Inc. (EHA). A higher percentage of the carbaryl-exposed workers (14.9%) showed depressed sperm counts (less than 20 million per ml) than did the control group (5.5%), but the difference is not considered statistically significant ($p = 0.0626$). Reproductive hormone levels in the carbaryl-exposed workers were "unremarkable" (normal).

19/ Of 49 original volunteers, 47 provided technically satisfactory semen samples. These same 49 volunteers later participated in the study conducted by Wyrobek et al. (1980).

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b. Rodent Data. In another study performed in Russia, Krylova and Denisova (1973) examined the process of spermatogenesis in the Mongolian tree creeper, a small rodent. Animals were trapped which inhabit an area that was sprayed with 85% Sevin wettable powder. The authors reported a reduction in the number of spermatozoa, spermatids, and spermatocytes statistically significant ($p<.001$) from the control group (tree creepers residing in an area that was not sprayed). However, the interpretation of the results in this article is tenuous because several factors -- e.g. the genetic variability of the animals, the duration in the amount of exposure to the chemical, and the age and health of the animals -- cannot be controlled in such a study.

Kitagawa et al. (1977) observed a reduction in the number of spermatogonia and spermatozoa in the testes when rats were orally administered 3 mg of carbaryl per rat per week for one year. Quantitated data were not presented, and the toxicity of carbaryl was not given at the doses used in these experiments.

Degraeve et al. (1976) observed an increase in the incidence of abnormal spermatozoa (no acrosome, abnormalities of flagellae) in the ducts deferens in carbaryl-treated male mice. These authors, however, did not demonstrate whether exposed mice showed dose-dependent increases in the induction of abnormal sperm.

Thomas et al. (1974) administered a single dose of radioactive (^{14}C) carbaryl (24 uCi/kg, 8.0 mg/kg) to mice. They found very small amounts of labeled carbaryl and/or its metabolites in the prostate, seminal vesicle, testes, seminal plasma, and epididymal fat. The radioactive counts were so low in this study that it is doubtful that these data are meaningful.

Shtenberg and Rybakova (1968) found a decrease in sperm motility in rats at 14 and 70 mg/kg/day after 6, 9, and 12 months of carbaryl treatment. This

effect on male fertility was dependent on the carbaryl concentration and duration of exposure.

In contrast, some investigators have reported no significant gonadal effects attributable to carbaryl. Dikshith et al. (1976) administered carbaryl (200 mg/kg for 3 days a week) orally to male rats for a period of 90 days. No histological charges were observed in the testes and epididymis. Also, these authors found no effects on the fertility of male rats. Weil et al. (1973) also observed no significant effects of carbaryl (10 mg/kg/day) on fertility in a 3-generation rat study.

Taking the full weight of evidence into account, the Agency has concluded that currently available data on carbaryl do not indicate that a rebuttable presumption on the basis of mutagenic effects is warranted at this time. Due to the weak mutagenic responses which have been measured, and due to the suggestive rather than conclusive nature of the evidence available as to the potential of carbaryl to reach the mammalian germinal tissue, the Agency believes that general exposure reduction methods typical of those already on many of the labels are appropriate and will be pursued prior to any further RPAR review.

D. Oncogenic Effects [11/]

40 CFR 162.11(a)3)(i)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) induce(s) "oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation or dermal exposure." The term "oncogenic" is defined in 40 CFR 162.3(bb) as "the property of a substance or mixture of substances to produce benign or malignant tumor formation in living animals." To determine whether or not carbaryl might pose an oncogenic hazard warranting a rebuttable presumption, the Agency has conducted a review of the available literature (CAG, 1977). A summary of the Agency's findings follows.

Innes et al. (1969) administered 4.64 mg/kg carbaryl in 0.5% gelatin daily by stomach tube to mice, strains X(C_{57}BL/6xC_3H/Anf) and Y(C_{57}BL/-6xAKR)F_1, beginning at 7 days of age and continuing until mice were 6 weeks old. (Carbaryl dosage was not adjusted according to weight gain during the 6-week period.) Vehicle groups and untreated control groups consisted of 18 animals each. Subsequently, the vehicle groups were fed a diet containing 14 ppm carbaryl, and the experiment was terminated when the mice were approximately 78 weeks of age. Treated mice showed no significant increase in the incidence of tumors over controls. It should be pointed out, however, that the 14 ppm dietary dosage of carbaryl was probably too low to be a firm

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11/ Only those data which are pertinent to oncogenicity as a possible adverse effect of carbaryl per se are evaluated here. The possibility of an oncogenic risk posed by formation of N-nitrosocarbaryl in the stomach will be addressed in a separate Agency review of nitroso compounds. (See Appendix B for a summary of current information concerning the carcinogenicity of N-nitroso-carbaryl.)

indicator of no oncogenic potential. The Agency points this out in view of 1) the circumstance that many of the carbaryl tolerances on food are as high as 12 ppm (Fed. Reg., special ed. [July 1, 1976]: 180.169), and 2) the fact that in a 1963 study conducted by the Mellon Institute (reviewed below) mice were fed 400 ppm carbaryl in the diet for 2 years and did not show any marked toxicity.

In a parallel experiment, Innes et al. (1969) administered a single subcutaneous dose of 100 mg carbaryl/kg in DMSO to weanling mice. Tumor incidence in treated animals was not significantly increased.

In a study conducted by the Mellon Institute (1963), groups of 48 male and 48 female CD-1 mice were fed diets containing 0.04% (400 ppm), 0.01% (100 ppm), and 0% carbaryl. Seventy-two of the 288 animals included in the study were given gross and histopathological examination. Of the other 216 animals, those which died during the initial 80-week period (50%) were subjected to gross examination, and tissues suspected of having pathology were examined histologically. Of the 65 animals allowed to live for 2 years, 9 males and 21 females were sacrificed at the end of the period, and no information was given on the remaining animals. No significant difference in tumor incidence between treated and untreated animals was observed in those mice sacrificed after 80 weeks, those which died during the 80-week period, or those sacrificed at the end of 2 years. Because this study is very seriously flawed, however, it cannot stand as a basis for any determination for or against the carcinogenic potential of carbaryl (CAG, 1977). Among numerous drawbacks, the most serious problems are 1) that no information is available concerning 93 of the 288 animals with which the experiment began, and 2) that for animals dying during the study, histopathology was carried out only on animals suspected of tumor growth and not on all animals.

In another experiment conducted by the Mellon Institute (1962), a mixture of 40 parts Sevin 85 sprayable powder (85% carbaryl) and 60 parts water was applied (schedule of application unspecified) on the skin of 36 mice for 30 months. None of the animals developed tumors. In the same experiment, methylcholanthrene (0.2 % solution in acetone) was applied to a group of 32 animals. All animals in this group developed tumors by the end of 12 months. Details concerning pathology and experimental procedure were not available for review, however.

Makovskaia et al. (1965) administered weekly doses of carbaryl (60 mg/kg in a 2% solution in sunflower oil) intraperitoneally to groups of line A and C_3HA mice. The experiment included 400 carbaryl-treated animals, 150 animals treated with urethane (200 to 1000 mg/kg intraperitoneally) as a positive control group and 100 animals in the untreated control group. Animals were sacrificed at 1, 3, 5, 9, 12, 15, 18, and 24 months after the test began, and several organs (lungs, kidneys, liver, heart, spleen, pancreas, thyroid, and adrenals) were reportedly examined histopathologically. Makovskaia et al. reported that "carbaryl treatment did not produce any new growth in lungs or liver," but their report failed to provide experimental details necessary for evaluation (CAG, 1977). No conclusion concerning the carcinogenic potential of carbaryl can be drawn from this study.

Carpenter et al. (1961) maintained groups of male and female CF-N rats on a mixture of Purina Chow and carbaryl (in concentrations of 0.04%, 0.02%, 0.01%, 0.005%, and 0.00%) for two years. Rats were 60 days old when the experiment began, and 20 rats were included in each group. Sixty-one of the

102 animals which died during the study, and 98 animals sacrificed at the end f the study, were examined histopathologically. Although female rats were found to have more pituitary tumors relative to males, no significant increase in tumors was found in treated groups relative to controls. The validity of Carpenter et al. is compromised, however, in that the study began not with newborn or weanling rats but with 60-day-old animals. Because they are known to be more sensitive to chemical carcinogenesis than are adults, newborn or weanling animals are preferred for purposes of cancer bioassays (CAG, 1977).

For purposes of their experiments with rats, Andrianova and Alekseev (1970) obtained carbaryl (reportedly 97.65% pure) from the Schelkovsky Chemical Plant. A group of 60 adult male rats (mongrels) was given 30 mg/kg carbaryl orally (as a water suspension) twice weekly by gavage for 22 months. This dose was approximately one-twentieth of the LD_{50} dose. Tumors, as well as organs suspected of having tumors, were examined histopathologically. Out of 12 rats alive in the treated group at the end of the experiment, four animals were found to have malignant tumors. All tumors were sarcomas (2 fibrosarcomas, 1 polymorphocellular sarcoma, and one osteosarcoma with various metastases). In another experiment, Andrianova and Alekseev (1970) introduced 20 mg of carbaryl (enclosed in a purified paraffin capsule weighing 250 mg) hypodermically in a group of 48 male rats. Tumors were discovered in 2 out of 10 rats that survived 22 months. In both animals, tumors (diagnosed as fibrosarcoma) were observed under the skin near the back, not near the site of the implant. Whether or not controls were similarly implanted with gelatin capsules without the chemical was unclear.

Andrianova and Alekseev used a group of 48 untreated rats as controls for both these experiments. Among controls, one fibrosarcoma was discovered after 1 months, at which time 46 of the original controls remained alive. The number of controls surviving the full 22 months of the experiment was not

given. The Agency points out that if all 46 controls survived to 22 months, the positive results of the feeding experiment would be significant. Andrianova and Alekseev state, however, that "the results concerning the average life span ... show that the pesticide doses selected were sufficiently well endured by the animals" (translation), and survival in experimental and control groups may have been comparable (CAG, 1977). Agency efforts to obtain further information necessary to evaluate the adequacy and significance of this Russian study have not been successful.

Two additional studies of the carcinogenic potential of carbaryl which were not included in the original review by EPA's Carcinogen Assessment Group (CAG, 1977) have since been evaluated by the Agency (CAG [Anderson] 1980a, 1980b). In a study performed by Shimkin et al. (1969), the carcinogenic potential of carbaryl was tested with the pulmonary tumor response in male A/He mice, 7 to 9 weeks old. Carbaryl was prepared by reacting its chloroformate with ammonia. The dosing vehicle was 99.9% pure tricaprylin. Intraperitoneal injections of 20 mg carbaryl/kg were made 3 times weekly for 4 weeks. The mice were sacrificed at 20 weeks after the final injection. Necropsies were performed, and lungs were examined microscopically. Untreated and vehicle-treated control animals were similarly maintained and evaluated.

Fifteen of an original 16 carbaryl-treated animals survived to 20 weeks. Upon sacrifice and examination, one lung tumor was found in each of 4 animals, and 2 lung tumors were found in each of 2 animals. Twenty-eight of an original 32 vehicle control animals survived to be sacrificed. Upon examination, 1 lung tumor was found in each of 6 animals, and 1 animal had 2 tumors. Thirty-one of an original 32 untreated control animals survived to be sacrificed. Upon examination, 1 lung tumor was found in each of 2 animals. The number of lung tumors per mouse in the carbaryl-treated, vehicle control, and untreated control groups was, respectively, 0.7, 0.3, and 0.1.

The tumor incidence observed by Shimkin et al. in carbaryl-treated mice 40%) was slightly higher than in the vehicle control group (25%). However, this difference is not statistically significant: $p > 0.05$, by the Chi-squared test. Moreover, Shimkin et al. in fact state that a slightly higher tumor incidence in vehicle-control mice (used as the standard comparison group in the above calculation) over untreated control mice is commonly observed in this strain. In the Agency's judgment, no conclusion about the potential human carcinogenicity of carbaryl can be made from this test(CAG [Anderson] 1980a). This particular bioassay is a short-term test with a mouse strain highly susceptible to lung tumor formation, and findings in this strain cannot be considered equivalent to a lifetime mouse bioassay. The essay is also flawed by other limitations: 1) the actual purity of the carbaryl test sample is not described, 2) the sample size of the groups is small, and 3) since the bedding was heat-treated absorbent cedar cubed wood, it is possible that terpenes or other substances in this material could have altered the rate of carbaryl metabolism in the mice.

A study has been reported by A.J. Triolo (1978) in a U.S. EPA document, which attempted to evaluate the ability of carbaryl to enhance the incidence of forestomach tumors in Ha/ICR female mice and lung tumors in A/J female mice treated with benzo(a)pyrene (BP). Dietary levels of carbaryl up to 2000 ppm did not increase the incidence of forestomach tumors in mice treated with 300 ppm benzo(a)pyrene in the diet for 12 weeks. In A/J mice given 3 mg benzo(a)pyrene *per os* on days 7 and 21, 1000 ppm carbaryl in the diet produced a statistically significant ($P<0.05$) increase in lung tumor incidence (18/18)

as compared to the BP-treated control (15/17). This increase is slight, however, and in a repeat experiment the incidence in the carbaryl-BP group (16/34) was similar to that in the BP group (16/31). The A/J mice were on study for 20 and 16 weeks, respectively, in these experiments. The Agency's conclusion with respect to this study is that a co-carcinogenic effect has not been conclusively demonstrated (CAG [Anderson], 1980b).

Based on the weight of evidence of available studies on carbaryl the Agency has concluded that a rebuttable presumption on the basis of oncogenic effects is not warranted at this time.

E. Neurotoxicity

As stipulated in 40 CRF 162.11(a)(3)(ii)(B), "a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) ... produces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

Three available studies concerning the neurotoxicity of carbaryl have been reviewed by the Agency. To test the neuromuscular degenerative potential of carbaryl as compared with that of triorthocresyl phosphate (TOCP), Carpenter et

al. (1961) administered single subcutaneous injections to 2-year-old moulting Rhode Island Red hens.[12/] These chemicals were mixed or suspended in lard melting at or below 37°C and administered at 0.25, 0.50, 1.0, 2.0, and 3.0 gram/kg dosage levels at concentrations of 25% to 40%. Thirteen hens were administered carbaryl, and 10 were administered TOCP thus diluted. In addition, undiluted TOCP was given to one chicken, and undiluted lard was given to two others. Five control chickens received no injections.

No adverse effects were observed at 1.0 g/kg or lower doses of carbaryl. Chickens administered 2.0 g/kg carbaryl showed leg weakness on the first or second day following dosing, and in only one case was the chicken nonambulant for 3 days. A nephrotoxic action was also observed in fowl which received 2.0 g/kg or larger injections of carbaryl. (Nephrotoxic action was apparent as a deposition of fine fat droplets within the epithelial cells lining the proximal tubules.) Necrosis was not present. In hens which received 3.0 g/kg carbaryl, lesions (focal loss of striation and fatty infiltration of

12/ The subcutaneous route was used because no satisfactory vehicle was then known by which to administer carbaryl intravenously.

gastrocnemius muscles) were observed. Such lesions were found at all levels in the examined after injection with TOCP, with the anomalous exception of the 2.5 g/kg dosage. Likewise at all dosage levels tested, animals injected with TOCP showed fatty deposition of a similar though more diffuse nature to that observed in fowl administered 2.5 g/kg or larger doses of carbaryl. At 2.5 g/kg, TOCP proved lethal in 7 to 10 days, with leg weaknesses observed in 3 of 4 cases only on the day of death. At all lower dosage levels, weakness was not apparent until the thirteenth or fourteenth day. Upon histopathological examination, slight evidence of demyelination was observed in three of the 10 TOCP-treated hens, whereas carbaryl-treated hens showed no signs of demyelination.

In screening tests for the production of paralytic effect in chicken hens, Gaines (1969) tested 30 organic phosphorous pesticides an 9 carbamate pesticides including carbaryl. The work of Gaines has been reviewed and validated for the Agency (Metcalf, 1977). Gaines (1969) administered carbaryl subcutaneously to hens at single doses of 800 and 1600 mg/kg. Prior to carbaryl treatment, the hens were treated with 15 mg/kg of atropine orally. All animals were observed for 24 days. The hens treated with 1600 mg/kg dose of carbaryl showed leg weakness within 24 hours, but all chickens recovered by day 24. The TOCP-treated animals developed paralysis after 14 days, and the paralysis continued until death.

Smalley et al. (1969) administered carbaryl at a dose of 150 mg daily for 30 and 90 days to one male pig and one female pig, respectively. In second experiment, one female and two males were given 150 mg/kg of carbaryl for 30 days, followed by 300 mg/kg/day for either 10 (males) or 57 (females)

retinal rays. Ataxia was observed in both experiments. Microscopic examination of skeletal muscle showed myodegeneration. The author reported that hydrochlorothiazide, a diuretic, reversed the signs of toxicity of carbaryl administration in chronic testing of pigs.

Based on currently available evidence as summarized above (Edwards, 1980), the Agency has concluded that carbaryl does not pose a neurotoxic hazard and that a rebuttable presumption on the basis of neurotoxicity is not warranted at this time.

F. Viral Enhancement

A recent study (Abrahamsen and Jerkofsky, 1980) which has come to the Agency's attention concerns viral enhancement as a possible adverse effect of human exposure to carbaryl. Abrahamsen and Jerkofsky investigated the effect of Sevin 4 oil on the replication of the human herpes virus varicella-zoster (VZ) in primary human embryonic lung (HEL) and WI-2 cell cultures. Complete Sevin 4 oil, its active ingredient (analytic grade carbaryl) and its "base oil plus inert ingredients" in sub-toxic concentrations, were tested for the ability to enhance the growth of VZ virus as measured by an infectious center assay. A 12- to 15 -fold increase in virus production by cells pretreated with Sevin 4 oil and carbaryl was observed. No enhancement was observed in base oil-treated cultures. Similar results were obtained when WI-2 cells were used. Viral enhancement appeared to be concentration-related in that decreasing concentrations of carbaryl brought decreases in viral enhancement. Experiments with herpes simplex virus type 1, however, showed no viral enhancement by Sevin 4 oil or any of its components. Abrahamsen and Jerkofsky suggest that the results of their work may be pertinent to studies of Reye's syndrome, or encephalopathy with fatty infiltration of the viscera "since published

epidemiological evidence has suggested a possible relationship between pesticide spraying, certain viral diseases including chickenpox or varicella, and the subsequent occurrence of Reye's syndrome" (Abrahamsen and Jerkofsky, 1980 [abstract]).

With regard to viral enhancement as a possible adverse effect of exposure to carbaryl, the Agency's determination at this juncture is that the work of Abrahamsen and Jerkofsky is preliminary in nature and that current data do not constitute a basis on which to conclude that carbaryl poses a human hazard in terms of viral enhancement. The Agency has therefore concluded that a rebuttable presumption is not warranted at this time.

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3. Oncogenic Effects. Based on the weight of existing evidence, the Agency has concluded that the current data base does not indicate that carbaryl poses an oncogenic hazard to humans. A rebuttable presumption on the basis of carbaryl-related oncogenic effects is therefore not warranted at this time.

3. Neurotoxicity. Based on available evidence, the Agency has concluded that carbaryl does not pose a human health hazard in terms of neurotoxic effects. A rebuttable presumption on the basis of neurotoxicity is therefore not warranted at this time.

5. Viral Enhancement. The Agency's determination at this juncture is that research into viral enhancement as a possible adverse effect of exposure to carbaryl is preliminary in nature and that current information does not constitute a basis on which to conclude that carbaryl poses a human hazard in this area. A rebuttable presumption on the basis of viral enhancement is therefore not warranted at this time.

6. Overview--Determining Considerations. Recognizing that the data base on any chemical is necessarily a continuum, the Agency's determination not to proceed with an RPAR action against carbaryl at this time takes into account a number of considerations in connection with the present toxicological picture of the pesticide. As has been pointed out, the current data base under review is extensive, more extensive than has ordinarily been the case for pesticides which have come under Agency review. This is particularly true for teratogenicity/fetotoxicity and mutagenicity, which are the toxicological areas of primary concern, and it is unlikely that resource-intensive RPAR procedures would surface data not already in the Agency's possession via other channels.

Although the current data base is extensive, risk data are not unequivocal, and study results, again in the areas of teratogenicity/fetotoxicity and mutagenicity, have been inconsistent. The current toxicological picture of carbaryl thus reflects a degree of uncertainty. It is in the face

[illegible]

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APPENDIX A -- REVIEW OF EXPERIMENTAL EVIDENCE ON THE MUTAGENICITY OF N-NITROSOCARBARYL

Carbaryl has been shown *in vitro* to react with sodium nitrite under acidic conditions (pH 1) to form N-nitrosocarbaryl (Eisenbrand et al., 1974). Because nitrite is present in human saliva and food products, the formation of nitrosocarbaryl in stomach physiology is possible, in view of the widespread use of carbaryl. Rickard (1979) demonstrated the *in vitro* formation of nitrosocarbaryl in the stomach of rats and guinea pigs. When guinea pigs were given either simultaneous intubation of carbaryl (1 umol) and sodium nitrite (1160 umol), or when these components were mixed with feed, approximately a 1.5% yield of nitrosocarbaryl was detected. The formation of this nitroso derivative was dependent on the amount of nitrite and the pH, and not particularly by the amount of carbaryl present. Increasing the amount of carbaryl from 0.025 to 2.5 umol did not increase the yield of the nitroso compound. In rats, the stomach pH (3.5-5.5) is higher than in guinea pigs (pH 1.5), and in that species a very low yield of nitrosocarbaryl was found (0.02%) at the same concentrations of nitrite and carbaryl.

Nitrosocarbaryl has been shown to be strongly mutagenic in bacteria. Blevins et al. (1977) found that the base-pair substitution sensitive Salmonella strains TA 100 and TA 1535 were reverted by this agent without metabolic activation. The reversion frequency in TA 100 was increased by approximately 1.6-fold at 1.15 ug/plate and 6-fold at 11.5 ug/plate, and TA 1535 by about 3-fold at 1.15 ug/plate and 76-fold at 11.5 ug/plate. Nitrosocarbaryl was not as active on the frameshift sensitive strains TA 98, TA 1537, and TA 1538. Marshall et al. (1976) found that nitrosocarbaryl increased the number of histidine-independent colonies of TA 1535 by approximately 6-fold at 0.5 ug/plate and by 367-fold at 50 ug/plate without metabolic activation. Marshall et al. also found nitrosocarbaryl to be slightly active (above 6-fold over background values) on the frameshift sensitive strains TA 1537 and TA 1538 at 50 ug/plate. Both Blevins et al. (1977) and Marshall et al. (1976) found that the mutagenic activity of nitrosocarbaryl was dose-related.

Elespuru and coworkers (1974) measured the induction to novobiocin resistance in Haemophilus influenzae. These authors found that nitrosocarbaryl was approximately an order of magnitude more potent than the mutagen N-methyl-N'-nitrosoguanidine (MNNG). In Escherichia coli nitrosocarbaryl was also more potent in the induction to arginine prototrophy than MNNG (Elespura et al., 1974). Uchiyama et al. (1975) found mutagenic activity as tested by the ability to cause reversion at the tryptophan locus in Escherichia coli (data not quantitated).

Generally, metabolic activation was not required for the mutagenic response of nitrosocarbaryl. For example, when Marshall et al. (1977) incorporated the S-9 fraction in the Salmonella assay, a decrease in mutagenic activity was observed. Greim et al. (1977), however, found an increase in mutagenicity after metabolic activation by mouse-liver microsomes.

Siebert and Eisenbrand (1974) reported that nitrosocarbaryl was active in causing mitotic gene conversion in Saccharomyces cerevisiae. Incubation for 2 hours on 1 ppm of nitrosocarbaryl increased the relative conversion frequency 3-fold for the ade-2 locus and 5-fold for the trp-5 locus, and at 30 ppm increases were 139-fold for the ade-2 locus and 885-fold for the trp-5 locus. In this study a dose-related effect was shown using 5 concentrations of nitrosocarbaryl. Regan et al. (1976) demonstrated that nitrosocarbaryl was able to induce DNA damage in culture human cells as measured by unscheduled DNA synthesis. In addition, by using methyl labeled [^{14}C] and ring labeled [^{3}H] nitrosocarbaryl, Regan et al. (1976) found that the ^{14}C label was associated with cellular DNA, whereas the ^{3}H label was not. Because nitrosocarbaryl has been observed to cause reversion of base-pair substitution sensitive strains (TA 100, TA 1535), these results suggest that the nitrosocarbaryl molecule was split and the resultant methyl group could alkylate DNA and cause base-pair substitution type mutations.

Ishidate and Odashima (1977) reported several chromosome aberrations (80% aberrant cells) in Chinese hamster cells 24 hours after exposure to nitrosocarbaryl (0.015 mg/ml). The toxicity of nitrosocarbaryl was not reported.

APPENDIX B -- REVIEW OF EXPERIMENTAL EVIDENCE ON THE CARCINOGENICITY OF N-NITROSOCARBARYL

As stated in Appendix A in connection with the mutagenicity of nitrosocarbaryl, carbaryl has been shown to react *in vitro* with sodium nitrite under acidic conditions to form nitrosocarbaryl, and formation of nitrosocarbaryl in stomach physiology is possible, given the presence of nitrite in human saliva and food products. N-nitrosocarbaryl is clearly a carcinogen in experimental animals, and this relates to the known carcinogenic properties of N-nitrosamines as a group.

Preussman et al. (1976) administered 130 mg/kg N-nitrosocarbaryl to "equal numbers" of 100-day-old male and female Sprague-Dawley rats, 200 g, twice weekly by gavage until death. Animals were given gross and histopathologic examinations. Twenty male and 20 female control animals were also on study. Hyperkeratoses, papillomas, and squamous cell carcinomas were found in the forestomach in 17 of 32 treatment rats. Mean induction time was 167 days. These anomalies were not present in control animals, although other types of tumors were found in 4 of the controls. The Agency notes that this study is flawed in that only one dose was used, but agrees that the results were clearly positive.

In a study performed by Lijinsky and Taylor (1976), twelve female Sprague-Dawley rats, 8 to 10 weeks old, were given 0.2 ml of a 0.11 M solution of nitrosocarbaryl in olive oil once weekly for 10 weeks to yield a total dose of 50 mg. Fifteen male Sprague-Dawley rats, 8 to 10 weeks old, were similarly treated with a 0.16 M solution twice weekly for 20 weeks to produce a total dose of 300 mg. Control animals were included in this study. Animals were examined for tumors with follow-up histopathologic evaluation on spontaneous death. In another experiment, a 19 umol solution of nitrosocarbaryl in acetone was applied twice weekly onto the backs of mice.

Females survived up to 110 weeks and males up to 90 weeks. Carcinoma in the forestomach was found in 9 females and 7 males, and papilloma in the esophagus or trachea was found in 1 male and 1 female. None of these tumor types was observed in control animals. Tumor metastases were found in treatment rats. Results were negative in the skin treatment experiment.

The Agency notes that small numbers of rats were used, which were dosed for a short period of time. However, this test was clearly positive. A lack of experimental details precludes a definite conclusion on the strengths and weaknesses of the skin treatment study.

In another study performed by Lijinsky and Taylor (1977), recrystallized technical grade carbaryl was administered to rats by gavage, either as a 30 mg/ml suspension in water or in 4.0% sodium nitrite ($NaNO_2$) solution. Three groups of 8 Sprague-Dawley female rats, 12 to 14 weeks old, were housed with males for mating during a weekend. One group was given 30 mg carbaryl

in water daily for 10 days during gestation days 4 to 18 (weekends omitted). One rat died during treatement, and 3 of those remaining gave birth to 9 female and 13 male pups. The second group was given 30 mg carbaryl in 4.0% $NANO_2$ solution on gestation days 4, 5, and 6. Six animals died, and one rat gave birth to 7 female and 3 male offspring. The third group was given 18 mg carbaryl in 4.0% $NANO_2$ on gestation days 14 to 18. None of these animals died, and 6 dams gave birth to 32 male and 32 female pups. In addition, a fourth group of 12 female rats was given the same treatment as the third group for 5 successive days. Whether or not this fourth group was mated was not specified, however, nor (if they were mated) is an evaluation of offspring shown. All adults and offspring were allowed to live until natural death except that moribund animals were sacrified. Necropsies were performed, and lesions and tumors examined histologically.

At least 50% of the dams and offspring reportedly survived to 100 weeks, and some survived 150 weeks. A statistically significant ($p < 0.05$) difference in tumor incidence between rats treated with carbaryl and those treated with carbaryl and nitrite was not evident. Data concerning all dams dosed with carbaryl and nitrite are summed together, however, and this makes a comparison with dams treated with carbaryl alone difficult. Nonetheless, adrenal, liver, and uterine tumors -- which were not evident in dams treated with carbaryl alone -- were detected in 2/22, 3/22, and 2/22 dams, respectively, given carbaryl plus nitrite. Among male offspring exposed during gestation days 14 to 18, breast tumors were diagnosed in 0/13

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pups in the carbaryl group and in 4/32 pups in the carbaryl plus nitrite group; pancretic tumors were observed in 1/13 pups in the carbaryl group and in 6/32 pups in the carbaryl plus nitrite group.

The Agency points out that the lack of concurrent control groups severely limits the usefulness of this study as an evaluation of the carcinogenic potential of carbaryl, and of carbaryl plus nitrite, under the experimental conditions described. With respect to statistical sensitivity, the sample sizes used in this study were rather small. The results of this study do not reflect a lifetime exposure of the experimental animals to carbaryl. Moreover, the effect which the administration of a suspension has on the actual absorption of carbaryl from the gastrointestinal tract needs to be considered and evaluated (CAG [Anderson], 1980).

In a study performed by Eisenbrand et al. (1975), a single subcutaneous injection of 1000 mg N-nitrosocarbaryl/kg in oil suspension was given to 8 male and 8 female Wistar rats. Vehicle controls were also evaluated. Another group of 37 male and 37 female Wistar rats (90 days old) received single gavage dosages of N-nitrosocarbaryl ranging from 200 to 1500 mg/kg. Untreated animals served as controls.

Fourteen rats treated subcutaneously died by day 450. All of these animals developed local tumors at the site of injection, which were diagnosed as polymorphic-cell sarcomas. Orally treated rats did not develop tumors by 21 months. Tumors were not found in control animals.

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The Agency notes that only single doses of N-nitrosocarbaryl were administered to evaluate carcinogenic potential, presumably with respect to the lifetime of the animals. Subcutaneous administration did not produce tumors beyond the site of administration, which makes these results difficult to interpret in relation to possible carcinogenic effects from other routes of administration.